

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT:	Zaghouani, H. et al.	)	ATTORNEY DOCKET:	0119742.00006
		)		
SERIAL NO.:	11/290,070	)	GROUP ART UNIT:	1644
		)		
FILED:	November 29, 2005	)	EXAMINER:	Ewoldt, Gerald R.
		)		
TITLE:	Methods and compositions for preventing the onset of type 1 diabetes ( <i>as amended</i> )			

**DECLARATION UNDER 37 C.F.R. § 1.132**

I, Habib Zaghouani, do hereby declare and say:

1. I am a citizen of the United States and my current residential address is 1608 Brookfield Manor, Columbia, Missouri, 65203.
2. I obtained my undergraduate degree in biochemistry from University of Paris, France in 1981. I obtained a Ph.D. in immunology from the University of Paris/Cancer Research Institute, France in 1987.
3. I am presently the J. Lavenia Edwards Chair in Pediatrics, Director, Center for Cellular and Molecular Immunology and Professor, Department of Molecular Microbiology & Immunology and Department of Child Health at the University of Missouri.
4. I have over one hundred publications and abstracts in the field of immunology. Please refer to the copy of my *curriculum vitae* in attached Appendix A for more details.
5. I am a named inventor on the '070 application as well as on related co-pending application serial numbers: 10/681,788; 11/510,411; and 11/425,084.
6. I have performed experiments examining the impact of administration, initiated at the pre-diabetic stage, of soluble Ig-GAD2 to NOD mice over a period of 56 weeks. Data are provided in attached Appendix B.
7. NOD mice were assessed for blood glucose beginning at week 12 of age. Those mice

that reached glucose levels of 160 – 250 mg/dl between week 14 to 25 received the following Ig-GAD2 regimen: 500 µg of soluble Ig-GAD2 i.p. daily for 5 days and then weekly injections thereafter for either 15 or 25 weeks. Blood glucose monitoring was performed during this period.

8. Overall, 100% of mice that became pre-diabetic at the age of 14 – 25 weeks and that were not treated with Ig-GAD2 progressed to diabetes (blood sugar level  $\geq 300$  mg/dl glucose) within 5 weeks after diagnosis of the pre-diabetic stage. Moreover, 60% of mice undergoing the 15-week treatment regimen were protected against diabetes throughout the 25 week post-hyperglycemia monitoring period. Interestingly, one mouse (Figure 1 B, left panel, open stars) progressed to diabetes by 5 weeks of treatment and 3 mice (Figure 1 B, plus, open diamond, and open pentagon) had similar disease manifestations shortly after interruption of the treatment.
9. When the regimen was extended to 25 weeks, 100% of the Ig-GAD2 treated animals were protected (Figure 1 A, right panel) and normoglycemia was restored in all mice (Figure 1 B, right panel). This status persisted throughout the duration of the study, which was terminated when the mice were 52 to 56 weeks of age.
10. Detailed histopathologic analysis from the mice was performed. While most of the islets in hyperglycemic and diabetic control mice exhibited intransulitis (Figure 2, panels 1, 2 and 3), the majority of islets in treated mice were not inflamed (Figure 2, panel 4) or had only mild periinsulitis (Figure 2, panels 5 and 6).
11. Overall, the histopathologic analysis indicated that treated mice had significantly greater number of islets when compared to both hyperglycemic and diabetic mice (Figure 3). The number of insulin-positive islets also increased from 14 per pancreas at the prediabetic stage to 29 per pancreas upon treatment with soluble Ig-GAD2. Analysis of islet infiltration scores among the different groups of mice indicated that the 15-week treatment group had a higher number of islets with periinsulitis (38% vs. 30%) or no insulitis (35% vs. 17%) relative to the hyperglycemic stage (Figure 4). On the other hand, the number of islets with severe- and mild-intransulitis were reduced in the treated versus hyperglycemic mice (8% and 19% vs. 22% and 31%, respectively) (Figure 4).

12. Surprisingly, in the 25-week treatment group, although the total number of islets was reduced to that of the hyperglycemic stage, most of these islets exhibited no (60%), peri- (28%) or mild intra- (12%) insulitis (Figure 4). Overall, the treatment with Ig-GAD2 led to a significant increase in the number of noninflamed ("healthy") islets that restored and maintained normoglycemia.
13. An experiment was performed to determine whether the healthy islets discussed above were a result of a regression of inflammation and/or regeneration of beta cells. To address this question, the treated mice were injected with the proliferation indicator 5-bromo-2'-deoxyuridine (BrdU), sacrificed and pancreatic sections were stained with anti-insulin and anti-BrdU antibodies and analyzed for BrdU incorporation and insulin production.
14. BrdU staining was visible in the highly proliferative luminal intestinal cells but these had no staining with anti-insulin antibody (Figure 5). Islets of non-diabetic 5-week old NOD mice were positive when stained with anti-insulin antibody, but did not incorporate BrdU, suggesting that these insulin-producing beta cells were not newly generated cells (Figure 6). Thus, under normal circumstances insulin production emanates from existing beta cells whose nuclei do not incorporate BrdU giving a minimal number of BrdU/insulin double-positive ( $\text{BrdU}^+/\text{insulin}^+$ ) beta cells (Figure 7). Sections from hyperglycemic mice showed very few insulin-producing beta cells and no BrdU incorporation (Figure 8) resulting in an insignificant number of  $\text{BrdU}^+/\text{insulin}^+$  beta cells (Figure 7). In contrast, islets from the 25-week treatment group showed beta cells that stained positive for insulin and were either BrdU negative (previously generated beta cells) or BrdU positive (newly generated beta cells) (Figure 9). Notably, the number of these insulin-producing regenerating beta cells was significantly increased in all five mice in which treatment restored normoglycemia (Figure 7).
15. Interestingly the total number of regenerating cells producing insulin ( $\text{Insulin}^+/\text{BrdU}^+$ ) was low and may not solely account for the restoration of normoglycemia. Insulin-positive / BrdU-negative islet cells may have also contributed to the control of blood glucose level and these likely represent a combination of newly regenerated and

previously existing beta cells. There were also numerous BrdU positive / insulin-negative islet cells that likely represent newly regenerating cells that are not yet producing abundant insulin (Figure 9).


16. Splenic cells from recovered mice were then stimulated with GAD2 peptide and assessed for both suppressive and inflammatory cytokines. The results indicated that although no measurable IL-4 or TGF $\beta$  was detected (not depicted), there was significant IFN $\gamma$  and IL-10 production by these cells relative to the control HEL peptide (Figure 10).
17. When *in vivo* cytokine neutralization was performed along with soluble Ig-GAD2 treatment, the recovery persisted with anti-IL 10 treatment but was nullified by removal of IFN $\gamma$  (Figure 11). These results indicate that IFN $\gamma$  (but not IL-10), contrary to IFN $\gamma$ 's well-defined inflammatory function, is likely involved in modulation of inflammation and restoration of normoglycemia.
18. Th17 cells represent a newly defined subset of pathogenic T cells whose development can be facilitated by TGF $\beta$  and IL-6 or interfered with by IFN $\gamma$  or IL-27. Because soluble Ig-GAD2 treatment induces IFN $\gamma$ , we sought to determine whether restoration of normoglycemia involves interference with IL-17 production. As such, an experiment was performed to assess whether IL-17 is produced by NOD T cells and, if so, to follow its pattern of secretion during disease progression.
19. IL-17 was evident upon IAA-seroconversion and increased significantly when the mice progressed to hyperglycemia and diabetes. Treatment with soluble Ig-GAD2 at the hyperglycemic stage significantly reduced the frequency of GAD2-specific IL-17 producing cells as measured by spot formation (data not shown). However, neutralization of IFN $\gamma$  by administration of anti-IFN $\gamma$  antibody along with Ig-GAD2 restored even higher frequency of Th17 cells which was likely caused by complete neutralization of IFN $\gamma$  (data not shown). It is therefore likely that the restoration of diabetes by neutralization of IFN $\gamma$  during treatment with Ig-GAD2 as seen in Figure 11 is actually caused by restoration of Th17.
20. At the time of filing of the '070 application T1D was suspected to involve multiple

autoantigens and the initiating antigen was unknown. As such, a person of ordinary skill in the art reading the literature at that time would have expected that induction of tolerance, if even possible, would require modulation of diverse T cell specificities through a combination of peripheral tolerance and bystander suppression.

21. It was known prior to filing of the '070 application that cross-linking of Fc receptors on target cells by antigen-antibody complexes could trigger the production of cytokines such as IL-10 which are important for down regulating T cells engaged to antigen presenting cells as well as neighboring T cells (*e.g.* bystander suppression).
22. Additionally, at the time of the '070 application, aggregation of Igs was known to confer effector functions associated with the Fc fragment without the need for complex formation.
23. In view of the foregoing, at the time the '070 application was filed, a person of ordinary skill in the art would not have believed that a *soluble* Ig-peptide chimera would be effective at treating T1D because such a person would not have expected a soluble Ig chimera to cross-link Fc receptors and stimulate cytokines (*e.g.* IL-10) by APCs so as to stimulate bystander suppression.
24. Surprisingly, we have found that soluble Ig-GAD2, despite the fact that it does not induce cross-linking of Fc receptors, delayed T1D when administered at the preinsulinitis stage and reversed diabetes when given at the prediabetic stage whereas aggregated Ig-GAD2 (which would be expected to cross-link Fc receptors and induce IL-10 secretion by APCs) did not delay diabetes when given after IAA seroconversion (unpublished data).
25. In my opinion, at the time of filing of the present application a person of ordinary skill in the art would not have expected that the presently claimed soluble Ig-GAD2 construct but not the aggregated Ig-GAD2 construct would delay T1D when administered after IAA seroconversion—a relatively advanced stage of disease.
26. In my opinion, a person of ordinary skill in the art, at the time of filing the present application, would not have expected that an antigen-specific single-epitope therapy such as soluble Ig-GAD2 would restore normoglycemia in hyperglycemic NOD mice.

27. It would have been particularly surprising to the person of ordinary skill in that art that soluble Ig-GAD2 was able to restore normoglycemia through an IL-10-independent mechanism. Such a person would certainly not have predicted that soluble Ig-GAD2 would delay T1D and reverse disease in pre-diabetic mice by inducing IFN $\gamma$  to suppress IL-17 which is contrary to IFN $\gamma$ 's well known inflammatory function.
28. Moreover, in my opinion, at the time of filing the present application a person of ordinary skill in the art would not have expected that treatment with the claimed soluble Ig-GAD2 construct would lead to an increase in the number of healthy, insulin positive pancreatic islet cells in NOD mice by comparison with controls.
29. I declare that all statements made herein of my own knowledge and are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

July 15, 2009  
Date

  
Habib Zaghouani, PhD

## APPENDIX A

### *Curriculum Vitae* Habib Zaghouani

#### **EDUCATION**

Ph.D.	1987	Immunology, University of Paris/Cancer Research Institute, Paris, France.
M.S.	1983	Immunology, University of Paris/Pasteur Institute, Paris, France.
B.S.	1981	Biochemistry, University of Paris, Paris, France.

#### **POSITIONS AND RESEARCH EXPERIENCE**

<b>2006-present</b>	Director, Center for Cellular and Molecular Immunology, The University of Missouri School of Medicine, Columbia, MO
<b>2006-present:</b>	J. Lavenia Edwards Chair in Pediatrics, the University of Missouri School of Medicine, Columbia, MO
<b>2006-present:</b>	Professor, Department of Child Health, the University of Missouri School of Medicine, Columbia, MO.
<b>2001-present:</b>	Professor, Department of Molecular Microbiology and Immunology, the University of Missouri School of Medicine, Columbia, MO.
<b>2000-2001:</b>	Associate Professor, Department of Microbiology, the University of Tennessee, Knoxville, Tennessee.
<b>1994-2000:</b>	Assistant Professor, Department of Microbiology, the University of Tennessee, Knoxville, Tennessee.
<b>1990-1994:</b>	Research Assistant Professor, Department of Microbiology, Mount Sinai School of Medicine, New York.
<b>1987-1989:</b>	Postdoctoral Fellow, Department of Microbiology, Mount Sinai School of Medicine, New York. Mentor: Dr. Constantin A. Bona.
<b>1983-1987:</b>	Graduate Research Assistant, Ph.D. candidate, Immunology, University of Paris/Cancer Research Institute, Paris, France. Mentor: Dr. Marc Stanislawski.
<b>1981-1983:</b>	Graduate Research Assistant, M.S. candidate, Immunology, Pasteur Institute, Paris, France. Director: Dr. Arthur Dony Strosberg.

## **RESEARCH GRANT SUPPORT**

### **A. Principal Investigator**

#### **Active**

- 1). **2RO1 NS 037406**, National Institutes of Health, March 2004 - February 2009. Modulation of autoreactive T cells. PI: Habib Zaghoulani.
- 2). **1RO1 DK 065748**, National Institutes of Health, April 2005-March 2008. Immune tolerance against type I diabetes in mice. PI: Habib Zaghoulani.
- 3). **2RO1 AI 48541**, National Institutes of Health, May 2006- April 2011. Regulation of neonatal immunity. PI: Habib Zaghoulani.
- 4). **1R21 AI 068746**, National Institutes of Health. July 2007 – June 2009. Mimotopes against type I diabetes. PI: Habib Zaghoulani.

#### **Pending**

- 1). **1RO1 NS057194-A2**, National Institutes of Health, April 2008 - March 2013. Regulation of autoimmune encephalomyelitis. PI: Habib Zaghoulani. **8.7 percentile**  
(a)
- 2). **2RO1 DK 065748-01**, National Institutes of Health, April 2008-March 2013. Immune tolerance against type I diabetes in mice. PI: Habib Zaghoulani. **35 percentile**

### **B. Co-investigator, Mentor, or Key Personnel**

#### **Active**

- T32 GM008396**, National Institute of General Medical Sciences (NIGMS), July 1991-June 2012. Molecular Basis of Gene Expression and Signal Processing. PI: Mark Hannink (Zaghoulani: Mentor).
- T32 RR007004**, National Institutes of Health, July 2005-June 2010, Postdoctoral Training in Comparative Medicine. PI: Craig Franklin (Zaghoulani: Mentor).
- T90 DK71510**, National Institutes of Health, September 2004 – August 2009. Bench and Back: Clinical bioterrorism training. PI: Mark Milanick (Zaghoulani: Mentor).
- R90 DK71510**, National Institutes of Health, September 2004 – August 2009. Bench and Back: Clinical bioterrorism training. PI: Mark Milanick (Zaghoulani: Mentor).



**KO8 AR048671**, National Institutes of Health, June 2005-April 2008, Cytokine regulation of collagen-induced arthritis. PI: Robert Ortman (Zaghouani: Mentor).

**1G20 RR021327**, National Institutes of Health, September 2004-August 2009. Equipment for the MU Life Sciences Center. PI: Lon Dixon, (Zaghouani: Key personnel).

**1 G20 RR019711**, National Institutes of Health, September 2004-August 2009. Renovation of MU Medical School Vivarium. PI: Lon Dixon. (Zaghouani: Key personnel).

**U19AT003264-01**, National Institutes of Health, September 2005 – August 2009. TICIPS: HIV/AIDS, Secondary Infections and Immune Modulation. Center grant. PI: William Folk (Zaghouani: Faculty Member).

**Research Foundation Grant**, Arthritis Foundation, April 2006 – May 2008. Synoviolin is a target for arthritis. PI: Deyu Fang (Zaghouani: Mentor).

### **C. Previous Support (PI: Zaghouani, H)**

1). **R21 AI 062796**, National Institutes of Health, July 2005-June 2007. Immune tolerance in the newborn mouse. Yearly direct cost \$150,000. PI: Habib Zaghouani. No cost extension 11/30/2007

2). **1RO1 AI48541**, National Institutes of Health, May 2001- April 2006. Regulation of neonatal immunity. Yearly direct cost: \$175,000. PI: Habib Zaghouani.

3). Astral Inc, October 2001- September 2004. Development of Approaches to Combat Autoimmunity. PI: Habib Zaghouani.

4). **RO1NS37406**, National Institutes of Health, January 2000- December 2004. Modulation of autoreactive T cells. PI: Habib Zaghouani

5). **RG2967B-3**, National Multiple Sclerosis Society, October 2002 – March 31, 2004 Down-regulation of encephalitogenic T cells. PI: Habib Zaghouani.

6). **RG2967A2/1**, National Multiple Sclerosis Society, April 99 - March 2002. Down-regulation of encephalitogenic T cells. PI: Habib Zaghouani.

7). Astral Inc: March 95 - July 2001. A novel approach to delete encephalitogenic T cells. PI: Habib Zaghouani.

8). **RG2778A1/1**, National Multiple Sclerosis, April 96 - March 1999. A deletional strategy for encephalitogenic T cells. PI: Habib Zaghouani.

9). Astral Inc.; September 97- August 99. Generation of human Ig chimeras carrying wild type or antagonist forms of myelin peptides. PI: Habib Zaghouani.

10). **1R41AI47496**, (STTR): National Institutes of Health, September 2000-August 2001. Treatment of EAE using a novel delivery system. . Co-PI: Habib Zaghouani.

## **TEACHING EXPERIENCE**

- 2004:** Microbiology 205 (Medical Microbiology) 3 credit hours, 8 lecture contact hours, 170 student, Spring semester, University of Missouri School of Medicine, Columbia.
- 2002-present:** Microbiology 304 (Immunology) 3 credit hours, 14 lecture contact hours, 30 students, Fall semester, Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia.
- 2002-present** Microbiology 407 (advanced Immunology) 4 credit hours, 9 lecture contact hours, 18 students, Spring semester, Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia.
- 2001-present:** Bio 4952, Undergraduate research, 3 credit hours, 1-2 students, Fall and Winter semesters
- 2001-present:** Bio 4950, Undergraduate research, 3 credit hours, 2-3 Students, Fall and Winter semesters
- 2001-present:** Direct Immunology Journal Club, 1hour/week all year around, 40 student, postdocs and faculty members
- 1995-2001:** Microbiology 430 (Immunology), 3 credit hours, 45 lecture contact hours, 100-120 students, Fall semester, Microbiology, The University of Tennessee, Knoxville.
- 1995-2001:** Co-direct Microbiology 602 (Microbial Pathogenesis Journal Club), 1 credit hour, 15 lecture contact hours, 10-15 students, Fall semester, Microbiology, The University of Tennessee, Knoxville.
- 1995-2001:** Co-direct Microbiology 603 (Immunology Journal Club), 1 credit hours, 15 lecture contact hours, 10-15 students, Spring semester, Microbiology, The University of Tennessee, Knoxville.
- 1995-2001:** Microbiology 401 (Undergraduate Research), 3 credit hours, 1-2 students per semester, Microbiology, The University of Tennessee, Knoxville.

- 1998:** Microbiology 630 (Topics in Immunology), 3 credit hours, 10 lecture contact hours, 20 students, Spring semester, (Seminar Series) Microbiology, The University of Tennessee, Knoxville.
- 1998-2001:** Microbiology 493 (Independent Study in Immunology), 6 students, 10 lecture contact hours, spring, Microbiology, The University of Tennessee, Knoxville.
- 1992-1994:** 600-level Immunology course, 3 credit hours, 6 lecture contact hours, 10 students, spring, Microbiology, Mount Sinai School of Medicine, New York.

### **HONORS AND AWARDS**

- 2006.** Speaker, Keystone Symposia on Tolerance Autoimmunity and Immune Regulation. March 21-26, 2006. Beaver Run Resort, Breckenridge, Colorado. Presentation title: Tregs for or against diabetes.
- 2004:** Research Equipment Award for the purchase of an ELISPOT Analyzer, Office of Research, The University of Missouri,
- 2003:** Keystone Symposia Scholarship (\$1,000) for poster presentation by Hyun-Hee Lee, a graduate student in the laboratory, the meeting was held in Snowbird, UT
- 2003:** Honorable citation for poster presentation by Randal Gregg, a graduate student in the laboratory. Life Science week, University of Missouri-Columbia.
- 2001:** Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.
- 2000:** Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.
- 2000:** Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of research Administration, The University of Tennessee, Knoxville.
- 1999:** Chancellor's nomination for Howard Hughes Medical Institute Assistant Investigator Appointment, The University of Tennessee, Knoxville.
- 1999:** Biological Equipment Award, Office of Research Administration/Science Alliance/Genome Science and Technology/Division of Biology, The University of Tennessee, Knoxville.
- 1999:** Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.
- 1999:** Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of research Administration, The University of Tennessee, Knoxville.

- 1998:** Science Alliance Research Excellence Award, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.
- 1998:** Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.
- 1997:** Biological Equipment Award, Office of Research Administration/Science Alliance/ Division of Biology/ Department of Microbiology, The University of Tennessee, Knoxville.
- 1997:** Exhibit, Performance, and Publication Expense Award, Faculty Senate Research Council and Office of Research Administration, The University of Tennessee, Knoxville.
- 1990:** Research Excellence Award, Alliance Pharmaceutical Corporation. San Diego, CA.
- 1987-1988:** Scientist Exchange Award (Postdoctoral Fellowship), French Cancer Society, Paris, France.
- 1984-1987:** Graduate Student Scholarship, French Cancer Society, Paris, France.

### **PROFESSIONAL SERVICE**

- 2007:** Chair, Block symposium, regulation of immune cell development and function, American Association of Immunologists, Miami, FL.
- 2006-2010:** Panel member, Hypersensitivity, Autoimmune and Immune-mediated Diseases (HAI) study section.
- 2006:** Chair, Block symposium, treatment of autoimmune disease, American Association of Immunologists, Boston, MA.
- 2006:** Review panel member, research proposals on Neurosciences, La Marato de TV3 Foundation, Catalan Agency For Health Technology Assessment And Research
- 2005:** Chair, Block symposium, Cytokines and autoimmunity, American Association of Immunologists, Experimental Biology Meeting, San Diego, CA.
- 2004:** Panel member, NIAID Biodefence Workshop, Immunization and Vaccination in Special Populations, Division of Allergy, Immunology and transplantation, NIH, Bethesda, MD

**2004:** Chair, Block symposium, Tolerance and regulation of autoimmunity, American Association of Immunologists, Experimental Biology Meeting, Washington DC.

**2004-present:** Adhoc Reviewer, TTT Study section, National Institutes of Health

**2004-present:** Adhoc Reviewer, HAI Study section, National Institutes of Health

**2003** Adhoc Reviewer, IMS Study Section, National Institutes of Health

**2003** Adhoc Reviewer, ALY Study Section, National Institutes of Health

**2003-present:** Member, Molecular Biology Program, University of Missouri-Columbia

**2003-present:** Member, Genetics Area Program, University of Missouri-Columbia

**2003-present:** Member, Veterinary Pathobiology Area Program, University of Missouri-Columbia

**2003-present** Scientific Consultant, Division of endocrinology and Diabetes, University of Missouri, Kansas City, MO

**2002-2004:** Scientific Consultant, Alliance Pharmaceutical, San Diego, CA.

**2001-present:** Member of The Graduate Student Recruitment Committee, Department of Molecular Microbiology and Immunology, The University of Missouri School of Medicine, Columbia.

**2000-2001:** Adhoc Reviewer, BM-1 Study Section, National Institutes of Health

**1992-2000:** Editorial board member: *Viral Immunology*

**1989-present:** Reviewer: Immunology Journals

**2000:** Guest Editor, International Review of Immunology

**2000-2001:** Chair, Graduate Student Advisory Committee, Genome, Science, and Technology program, Oak Ridge National Laboratories and The University of Tennessee, Knoxville.

**1995-2001:** Member of The Graduate Student Recruitment Committee, Department of Microbiology, The University of Tennessee, Knoxville.

**1998:** Member of Faculty Search Committee, Department of Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville.

**1999:** Panel Member: NIH/NCI, Small Business Innovation Research (SBIR)/Small Business Technology Transfer (STTR) Grant program.

Flexible system to advance innovative research for cancer drug discovery by small business panel.

## **PROFESSIONAL MEMBERSHIP**

**2006-present:** Member of the Henry Kunkel Society  
**1998-present:** Member of the Society for Neuroscience  
**1992-present:** Member of the American Association for the Advancement of Science.  
**1992-present:** Member of the American Association of Immunologists.

## **PUBLICATIONS**

### **Manuscripts published in peer-review journals**

1. Bot, A., D. Smith, B. Phillips, S. Bot, C. Bona, and H. Zaghoulani. (2006). Immunologic control of tumors by *in vivo* FcγR-targeted antigen loading in conjunction with dsRNA-mediated immune modulation. J. Immunol. 176:1363-1374.
2. 58. Caprio-Young, J., J. J. Bell, H-H. Lee, J. S. Ellis, D. M. Nast, G. Sayler, B. Min, and H. Zaghoulani. (2006). Neonatally Primed Lymph Node but not Splenic T Cells Display a Gly- Gly Motif Within the T Cell Receptor Beta Chain Complementarity Determining Region 3 (CDR3) That Controls Affinity and Lymphoid Organ Retention. J. Immunol. 176:357-364.
3. Yu, P., R. K. Gregg, J. J. Bell, J. S. Ellis, R. Divekar, H-H Lee, R. Jain, H. Waldner, J. C. Hardaway, M. Collins, V. K. Kuchroo, and H. Zaghoulani. (2005). Specific T regulatory cells (Tregs) display broad suppressive functions against experimental allergic encephalomyelitis upon activation with cognate antigen. J. Immunol. 174:6772-6780.
4. Gregg, R. K., J. J. Bell, H-H. Lee, R. Jain, S. J. Schoenleber, R. Divekar, and H. Zaghoulani (2005). IL-10 diminishes CTLA-4 expression on islet-resident T cells and sustains their activation rather than tolerance. J. Immunol. 174: 662-670.
5. Gregg, R. K., R. Jain, S. J. Schoenleber, R. Divekar, J. J. Bell, H-H. Lee, P. Yu, and H. Zaghoulani. (2004). A sudden decline in active membrane-bound TGFβ impairs both T regulatory cell function and protection against autoimmune diabetes. J. Immunol. 173:7308-7316.
6. Li, L, H-H. Lee, J. J. Bell, R. K. Gregg, J. S. Ellis, A. Gessner, and H. Zaghoulani. (2004). IL-4 Utilizes an Alternative Receptor to Drive Apoptosis of Th1 Cells and Skews Neonatal Immunity Towards Th2. Immunity. 20: 429-440.
7. Bell, J. J., B. Min, R. Gregg, H-H. Lee, and H. Zaghoulani. (2003). Break of neonatal Th1 tolerance and exacerbation of experimental allergic encephalomyelitis by interference with B7 costimulation. J. Immunol. 171:1801-1808.

8. Legge, K. L., Gregg, R. K., Maldonado-Lopez, R., Li, L., Caprio, J. C., Moser, M., and Zaghouani, H. (2002). On the role of dendritic cells in peripheral T cell tolerance and modulation of autoimmunity. J. Exp. Med. 196:217-227.
9. Pack, C. D., Cestra, A. E., Min, B., Legge, K. L., Li, L., Caprio, J. C., Bell, J. J., Gregg, R. K., and Zaghouani, H. (2001). Neonatal exposure to antigen primes the immune system to develop responses in various lymphoid organs and promotes bystander regulation of diverse T cell specificities. J. Immunol. 167:4187-4195.
10. Li, L., Legge, K. L., Min, B., Bell, J. J., Gregg, R., Caprio, J. and Zaghouani, H. (2001). Neonatal immunity develops in a transgenic TCR transfer model and reveals a requirement for elevated cell input to achieve organ-specific responses. J. Immunol. 167:2585-2594.
11. Min, B., Legge, K. L., Li, L., Caprio, J. C., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2001). Defective expression of CD40L undermines both IL-12 production by antigen presenting cells and up-regulation of IL-2 receptor on splenic T cells and perpetuates INF $\gamma$ -dependent T cell anergy. J. Immunol. 166:5594-5603.
12. Day, R. B., Okada, M., Ito, Y., Tsukada, K., Zaghouani, H., Shibuya, N., and Stacey, G. (2001). Binding site of chitin oligosaccharides in the soybean plasma membrane. Plant. Phys. 126:1-12.
13. Legge, K. L., Min, B., Caprio, J. C., Li, L., Gregg, R. K., Bell, J. J., and Zaghouani, H. (2000). Coupling of peripheral tolerance to endogenous IL-10 promotes effective modulation of myelin-activated T cells and ameliorates experimental allergic encephalomyelitis. J. Exp. Med. 191:2039-51.
14. Anderson, A. C., Nicholson, L. B., Legge, K. L., Turchin, V., Zaghouani, H., and Kuchroo, V. K. (2000). High frequency of auto-reactive myelin proteolipid protein (PLP)-specific T cells in the periphery of naïve mice: mechanisms of selection of the self-reactive repertoire. J. Exp. Med. 191:761-770.
15. Min, B., Legge, K. L., Caprio, J. C., Li, L., Gregg, R., and Zaghouani, H. (2000). Differential control of neonatal tolerance by antigen dose versus extended exposure and adjuvant. Cell. Immunol. 200 :45-55.
16. Legge, K. L., Min, B., Pack, C. D., Caprio, J. C., and Zaghouani, H. (1999). Differential presentation of an altered peptide within fetal central and peripheral organs supports an avidity model for thymic T cell development and implies a peripheral re-adjustment for activation. J. Immunol. 162:5738-46.
17. Min, B., Legge, K. L., Pack, C. D. and Zaghouani, H. (1998). Neonatal exposure to a self peptide-Ig chimera circumvents the use of adjuvant and confers resistance to autoimmune disease by a novel mechanism involving IL-4 lymph node deviation and INF $\gamma$ -mediated splenic anergy. J. Exp. Med. 188:2007-17.
18. Legge, K. L., Min, B., Cestra, A.E., Pack, C. D., and Zaghouani, H. (1998). T cell receptor agonist and antagonist exert in vivo cross-regulation when presented on immunoglobulins. J. Immunol. 161:106-11.

19. Legge, K. L., Min, B., Potter, N.T., and Zaghouani, H. (1997). Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or protein requiring endocytic processing. J. Exp. Med. 185:1043-53.
20. Brumeanu, T-D, Dehazya, P., Wolf, I., Bot, A., Bona, C., and Zaghouani, H. (1996). Engineering of double antigenized Igs carrying B and T cell epitopes. Immunotechnology 2:85-95.
21. Brumeanu, T-D., Zaghouani, H., and Bona, C. (1995). Purification of antigenized immunoglobulins derivatized with monomethoxypolyethylene glycol. J. Chromatogr. 696:219-25.
22. Brumeanu, T-D., Zaghouani, H., Elahi, I., Daian, C. and Bona, C. (1995). Derivatization with monomethoxypolyethylene glycol of Igs expressing viral epitopes obviates adjuvant requirement. J. Immunol. 154:3088-95.
23. Zaghouani, H., Anderson, S., Sperber, K. E., Daian, C., Kennedy, R. C., Mayer, L. and Bona, C. (1995). Induction of antibodies to the human immunodeficiency virus type 1 by immunization of baboons with immunoglobulin molecules carrying the principal neutralizing determinant of the envelope protein. Proc. Natl. Acad. Sci. USA. 92:631-35.
24. Bona, C., Brumeanu, T-D and Zaghouani, H. (1994). Immunogenicity of microbial peptides grafted in self immunoglobulin molecules. Cell. Mol. Biol. 40 (suppl):21-30.
25. Brumeanu, T-D., Swiggard, W. J., Steinman, R. M., Bona, C., and Zaghouani, H. (1993). Efficient loading of identical peptide onto class II molecules by antigenized immunoglobulin and PR8 virus. J. Exp. Med. 178:1795-99.
26. Brumeanu, T-D., Kohanski, R., Bona, C., and Zaghouani, H. (1993). A sensitive method to detect defined peptide among those eluted from murine MHC class II molecules. J. Immunol. Meth. 160:65-71.
27. Kuzu, Y., Kuzu, H., Zaghouani, H., and Bona, C. (1993). Priming of CTLs at various stages of ontogeny with transfectoma cells expressing a chimeric Ig heavy chain gene bearing an influenza virus nucleoprotein. International. Immunol. 5:1301-07.
28. Zaghouani, H., Kuzu, Y., Kuzu, H., Swigard, W., Steinman, R., and Bona, C. (1993). Contrasting efficacy of presentation by major histocompatibility complex class I and class II products when peptides are administered within a common protein carrier, self immunoglobulin. Eur. J. Immunol. 23:2746-50.
29. Penney, C. L., Ethier, D., Dionne, G., Nixon-George, A., Zaghouani, H., Michon, F., Jennings, H., and Bona, C. (1993). Further studies on the adjuvanticity of stearyl Tyrosine and ester analogues. Vaccine. 11:1129-1134.
30. Kuzu, H., Kuzu, Y., Zaghouani, H., and Bona, C. (1993). In-vivo priming effect during various stages of ontogeny of an influenza virus nucleoprotein derived peptide. Eur. J. Immunol. 23:1397-1400.



31. Zaghouani, H., Steinman, R., Nonacs, R., Shah, H., Gerhard, W. and Bona, C. (1993). Efficient presentation of a viral T helper epitope expressed in the CDR3 region of a self immunoglobulin molecule. Science. 259:224-27.
32. Shengqiang, L., Polonis, V., Isobe, H., Zaghouani, H., Guinea, R., Moran, T., Bona, C., and Palese, P. (1993). Chimeric influenza virus induces neutralizing antibodies and cytotoxic T cells against human immunodeficiency virus type 1. J. Virol. 67:6659-66.
33. Hall, B., Zaghouani, H., Daian, C. and Bona, C. (1992). A single amino acid mutation in CDR3 of the 3-14-9 light chain abolished expression of the IDA 10 defined idiotype and antigen binding. J. Immunol. 149:1605-12
34. Nixon, A., Zaghouani, H., Penney, C. L., Lacroix, M., Dionne, G., Anderson, S., Kennedy, R. C. and Bona, C. A. (1992). Adjuvanticity of stearyl tyrosine on the antibody response to peptide 503-535 from HIV gp160. Viral. Immunol. 5:141-50
35. Zaghouani, H., Krystal, M., Kuzu, H., Moran, T., Shah, H., Kuzu, Y., Schulman, J. and Bona, C. (1992). Cells expressing a heavy chain immunoglobulin gene carrying a viral T cell epitope are lysed by specific cytolytic T cells. J. Immunol. 148:3604-09.
36. Zaghouani, H., Goldstein, D., Shah, H., Anderson, S., Lacroix, M., Dionne, G., Kennedy, R. C. and Bona, C. (1991). Induction of antibodies to the envelope protein of the human immunodeficiency virus by immunization with monoclonal anti-idiotypes. Proc. Natl. Acad. Sci. USA. 88:5645-49.
37. Kaushik, A., Mayer, R., Fidanza, V., Zaghouani, H., Lim, A., Bona, C. and Dighiero, G. (1990). LY-1 and V-gene expression among hybridomas secreting natural autoantibody. J. Autoimmunity. 3:687-700.
38. Mayer, R., Zaghouani, H., Usuba, O. and Bona, C. (1990). The LY-1 gene expression in murine hybridomas producing autoantibodies. Autoimmunity. 6:293-305.
39. Bonilla, F. A., Zaghouani, H., Rubin, M. and Bona, C. (1990). VK gene usage, idiotype expression, and antigen binding among clones expressing the VHX24 gene family derived from naive and anti-id immune Balb/c mice. J. Immunol. 146:616-22.
40. Fidanza, V., Mayer, R., Zaghouani, H., Diliberti, M. A., and Bona, C. (1990). Autoantibodies, LY-1 and immunoglobulin V gene expression in hybridomas obtained from young and old NZB mice. Arthritis & Rheumatism. 33:711-23.
41. Zaghouani, H., Bonilla, F. A., Meek, K. & Bona, C. (1989). Molecular basis for expression of the A48 regulatory idiotype on antibodies encoded by immunoglobulin variable region genes from various families. Proc. Natl. Acad. Sci. USA. 86:2341-45.
42. Zaghouani, H., Fidanza, V. and Bona, C. (1989). The significance of idiotype-anti-idiotype interactions in the activation of self reactive clones. Clin. Exp. Rheumatology. 7/S-3:S19-25.

43. Pinter, A., Honnen, W. J., Tilley, S. A., Bona, C., Zaghouani, H., Zolla-Pazner, S. and Gorny, M. (1989). Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. 63:2674-79.
44. Zaghouani, H., Pene, J., Rousseau, V. and Stanislawski, M. (1988). A new strain specific cross-reactive idiotypic with possible regulatory function expressed on Balb/c anti- $\alpha(1-3)$  dextran antibodies. J. Immunol. 140:3844-50.
45. Zaghouani, H., and Stanislawski, M. (1987). Regulation of the response to  $\alpha(1-3)$  dextran: An anti-dextran associated idiotope of Balb/c mice is also expressed on A/J anti-NIP antibodies. Mol. Immunol. 24:1237-42.
46. Bara, J., Gautier, R., Zaghouani, H. and Decans, C. (1986). Monoclonal antibodies against oncofetal mucin M1 antigens associated with precancerous colonic mucosae. Cancer Res. 46:3983-89.
47. Pene, J., Rousseau, V., Zaghouani, H., Paroutaud, P., Strosberg, D. and Stanislawski, M. (1986). Monoclonal anti- $\alpha(1-3)$  dextran antibodies of Igha Balb/c and Igh<sup>b</sup> C.B20 mice display striking similarities. J. Immunol. 137:2319-24.
48. Pene, J., Bekkoucha, F., Desaymard, C., Zaghouani, H., and Stanislawski, M. (1983). Induction of a cross-reactive idiotypic dextran-positive antibody response in two IghC<sup>b</sup> mouse strains treated with anti-J558 cross-reactive idiotypic antibodies. J. Exp. Med. 157:1573-93.

### **Book Chapters and Reviews**

1. Phillips W. J., D. J. Smith, C. A. Bona, A. Bot, and H. Zaghouani. (2005). Recombinant immunoglobulin-based epitope delivery: a novel class of autoimmune regulators. Int Rev Immunol. 24:501-517.
2. Legge, K. L., J. Jeremiah Bell, L. Li, R. Gregg, J.C. Caprio, and H. Zaghouani. (2001). Multi-modal antigen specific therapy for autoimmunity. Intl. Rev. Immunol. 20: 593-611.
3. Min, B., Legge, K. L., Li, L., Caprio, J. C., Pack, C. D., Gregg, R., McGavin, D., Slauson, D., and Zaghouani, H. (1999). Neonatal tolerant immunity for vaccination against autoimmunity. Intl. Rev. Immunol. 2000 : 247-264.
4. Zaghouani, H., Kuzu, Y., Kuzu, H., Mann, N., Daian, C., and Bona, C. (1993). Engineered immunoglobulin molecules as vehicles for T cell epitopes. Int. Rev. Immunol. 10:265-77.
5. Zaghouani, H., and Bona, C. (1992). Stimulation of lymphocytes by anti-idiotypes bearing the internal image of viral antigens. In T Lymphocytes Structure, Function, Choices ( eds, Celada, F., and Pernis, B). NATO ASI SERIES, Series A: Life Sciences 233: 121-23.
6. Zaghouani, H., Hall, B., Shah, H. and Bona, C. (1991). Immunogenicity of synthetic peptides corresponding to various epitopes of the human immunodeficiency virus

envelope protein. In *Adv. Exp. Med. Biol.* (ed, Atassi, Z ). Plenum Press, New York. 303: 53-62

7. Mayer, R., and Zaghouani, H. (1991). Molecular studies on the contribution of the LY-1 B cell subset to self-reactivity. In *Molecular Immunobiology of Self Reactivity*. Immunology series. (eds, Bona, C. & Kaushik, A. ) Marcel Dekker Publisher, New York. . 55: 61-79
8. Bonilla, F. A., Zaghouani, H. and Bona, C. (1990) Patterns of idiotypic similarity and their structural bases among antibodies specific for foreign and self antigens. In *Idiotypic in Biology and Medicine*. (eds, Carson, D. A., Chen, P. P. and Kipps, T. J.). Prog. Chem. Immunol. Basel, Karger. 48:49-62.
9. Mayer, R., Zaghouani, H., Kaushik, A., Kasturi, K., Fidanza, V. and Bona, C. (1990). The expression of LY-1 and immunoglobulin variable gene families in hybridomas producing autoantibodies of various specificities. In *The Molecular Aspects of Autoimmunity*. (eds, Farid, N. R. and Bona, C.A.). Academic Press, PP 1-27.
10. Zaghouani, H., Victor-Kobrin, C., Barak, Z., Bonilla, F.A. and Bona, C. (1988). Molecular profile of monoclonal antibodies expressing the A48 regulatory idiotype and having distinct antigenic specificities. *Ann. New York. Acad. Sci.* 546:248-50.
11. Pene, J., Zaghouani, H., and Stanislawski, M. (1984). Regulation of the response to  $\alpha(1-3)$  dextran in IgH<sup>C<sub>b</sub></sup> mice. *Ann. New York. Acad. Sci.* 814:296-304.

### Abstracts

About 50 abstracts were published in the last 5 years

### PATENTS

1994. Patent # 5,969,109. chimeric antibodies comprising antigen binding sites and B and T cell epitopes, Constantin Bona and **Habib Zaghouani**. Issued . Mount Sinai School of Medicine, New York, NY.
1997. Patent # 08/779,767. Compound, compositions and methods for the endocytic presentation of immunosuppressive factors, **Habib Zagouani**. Issued. The University of Tennessee, Knoxville, TN.
2003. Multi-modal strategy for effective suppression of diabetes, **Habib Zaghouani**. Pending (#60/371,663). The University of Missouri, Columbia, MO.

# APPENDIX B

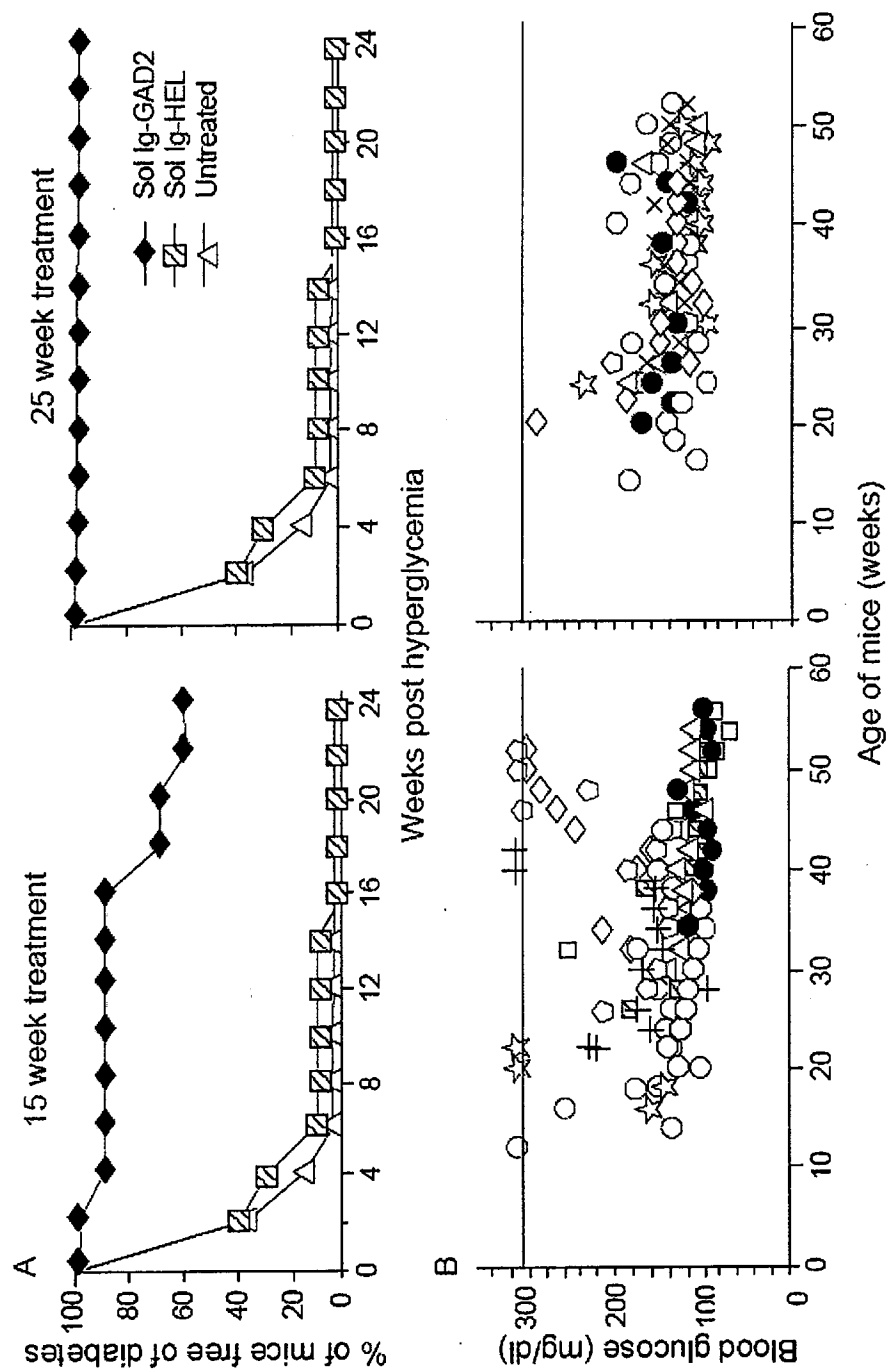


Figure 1

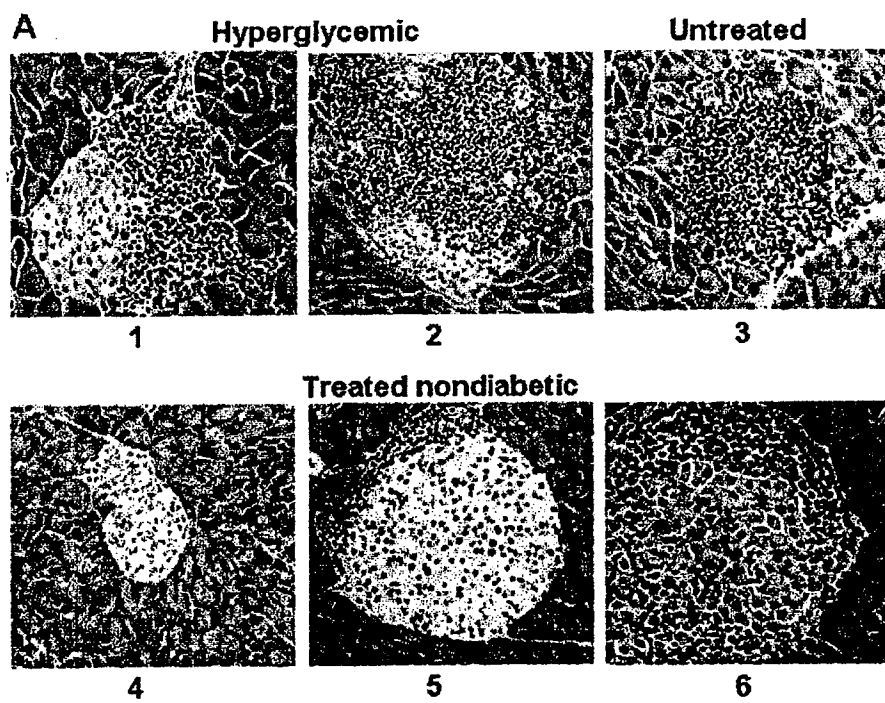


Figure 2

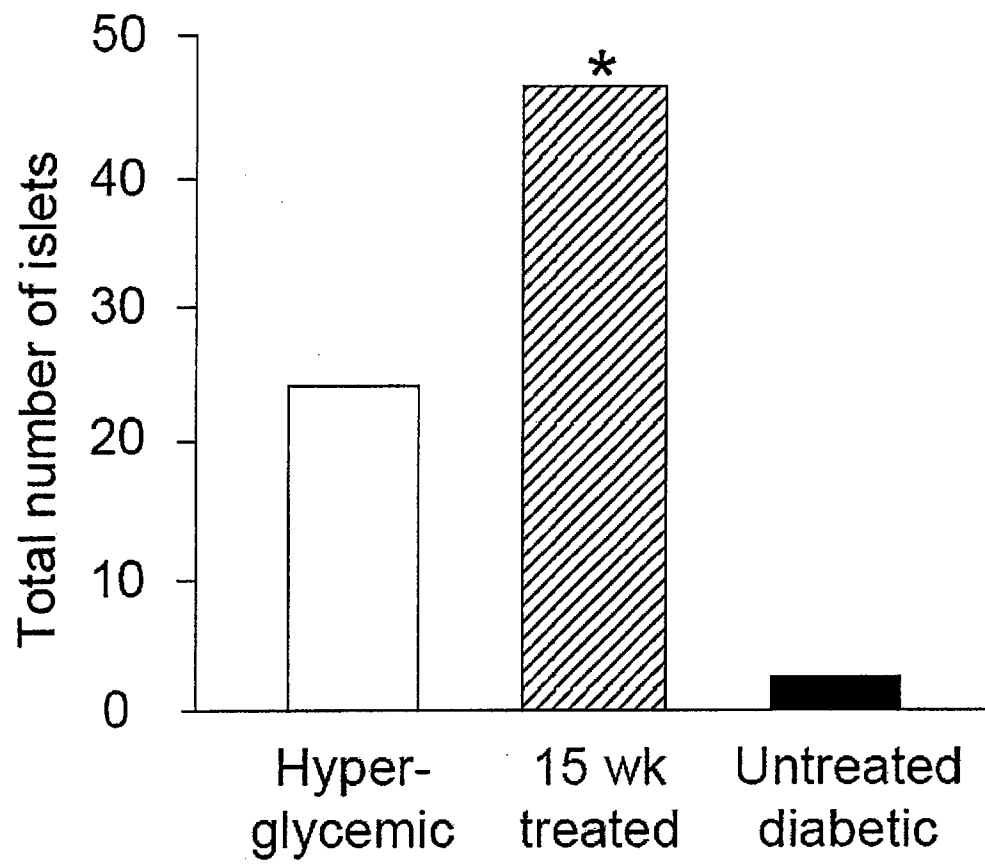


Figure 3

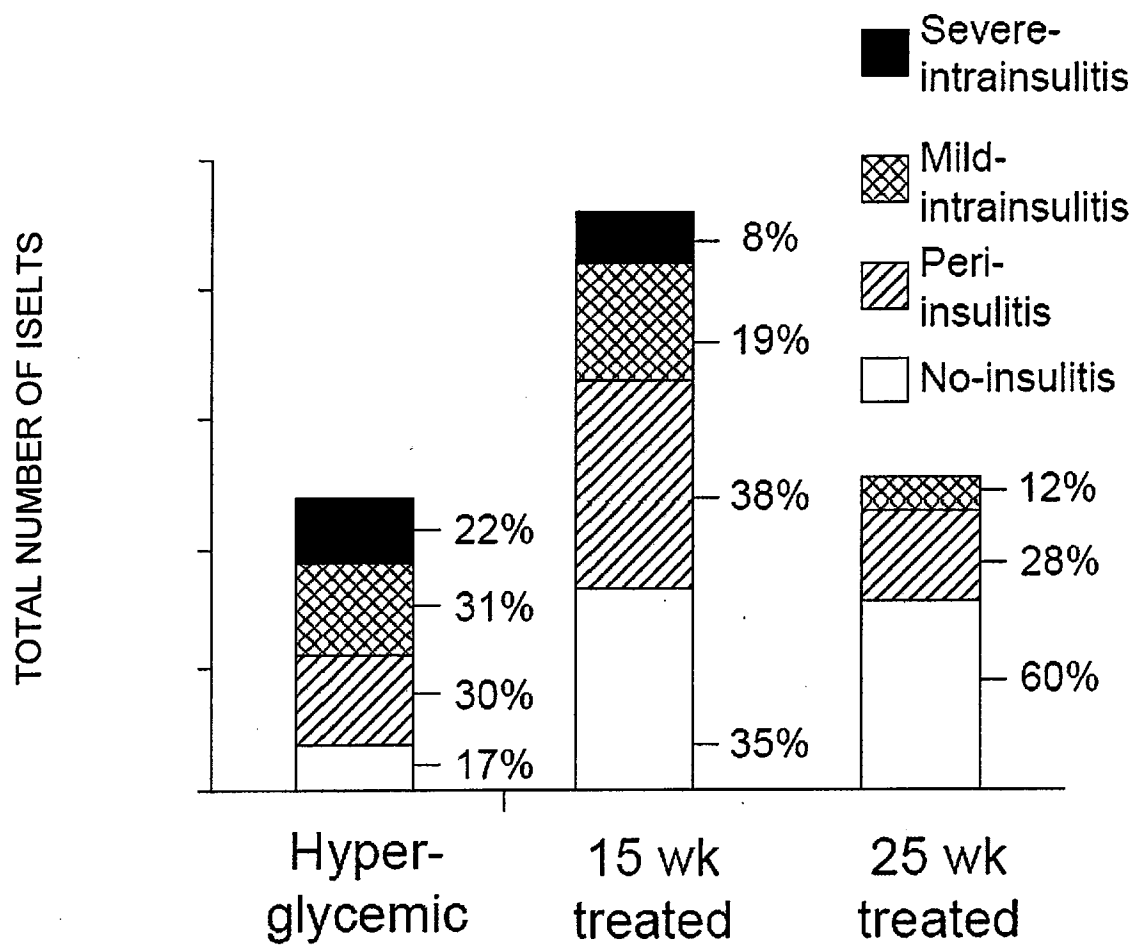


Figure 4

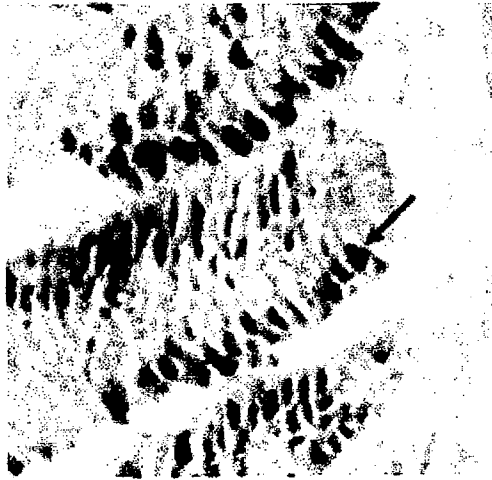


Figure 5





**Normal**

Figure 6

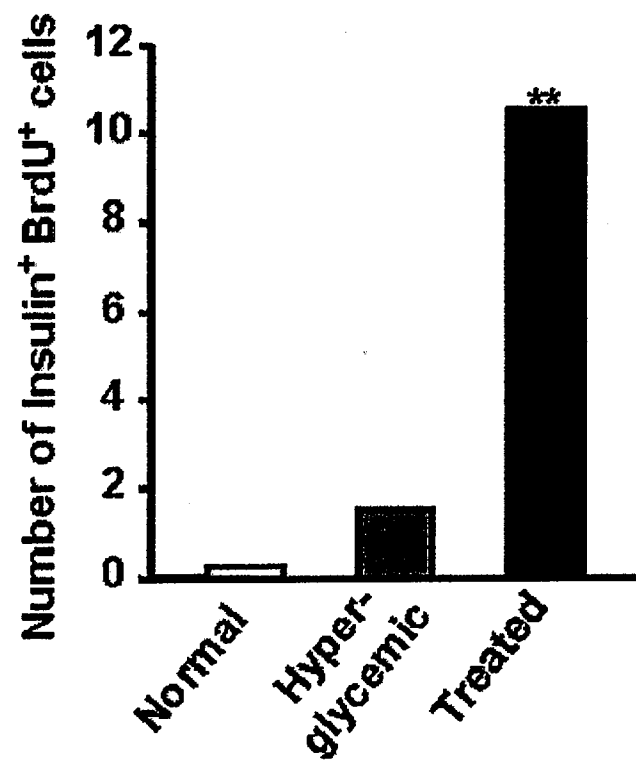
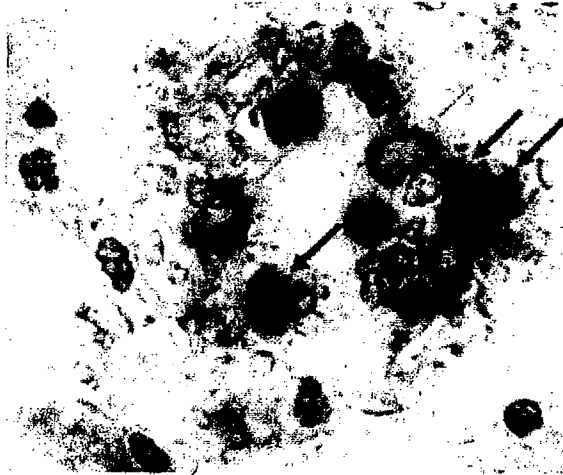


Figure 7



**Hyperglycemic**

Figure 8



**Treated**

Figure 9

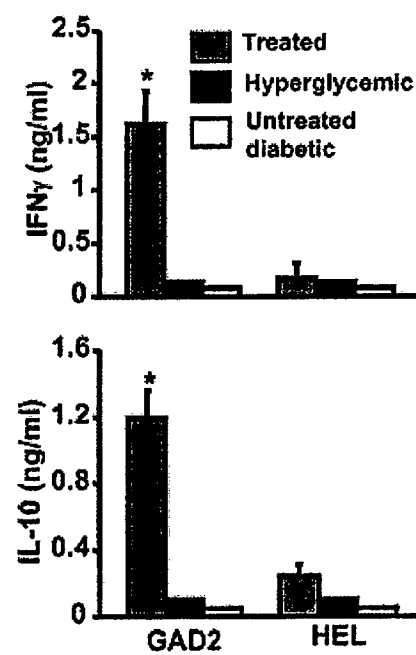


Figure 10

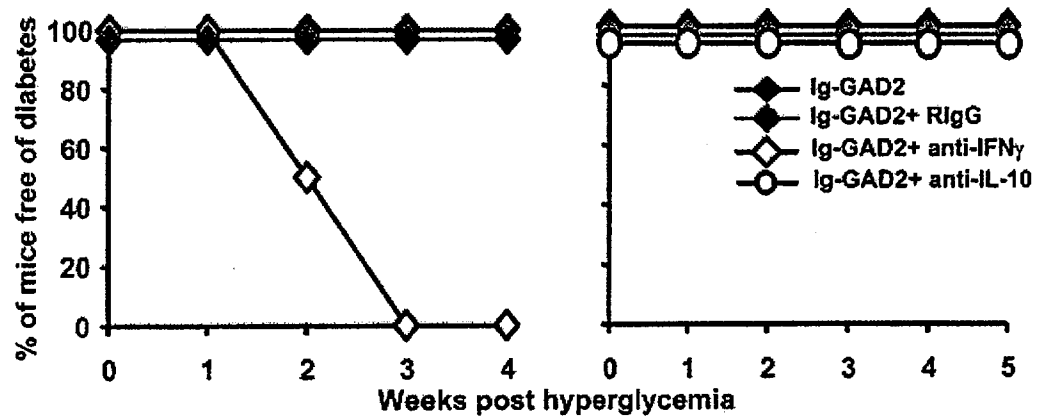


Figure 11